Histological study about *Leishmania infantum* infection on canine inflammatory bowel disease

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INTRODUCTION

The inflammatory bowel disease (IBD) is an idiopathic inflammatory disorder that affects gastrointestinal tract (GIT). The histopathological evaluation shows an inflammation in the colonic mucosa: lymphoplasmacytic colitis and histiocytic colitis. A pathogen or infectious agent could be a possible cause of IBD such as *Leishmania infantum*. The **main aim** of this study was to clarify if *L. infantum* infection would be a possible cause of IBD.

MATERIAL AND METHODS

1. Database selection

Reports search of compatible cases
- Diagnet application of Servei de Diagnòstic de Patologia Veterinària (SDPV).

Selection of cases
- Lymphoplasmacytic or histiocytic enteritis / colitis / enterocolitis.

Table with report's information
- Information: breed, age, sex (castrated / not castrated), clinical signs, type of sample, histological diagnosis, etc.

Selection criteria
- Compatible clinical signs
- Colon samples
- Inflammatory lymphoplasmacytic or histiocytic lesion
- Samples obtained by endoscopy
- 2007 or later

2. Immunohistochemistry for *Leishmania*

Leishmania IHC: Dako Autostainer Plus standard protocol, using a rabbit non-commercial polyclonal antiserum for *L. infantum*.

Samples study
- Examination of the samples (IHC & H-E) to assess the presence of amastigotes and type and intensity of inflammation.
- Counting 5 fields 40x to assess the degree of parasitism.

SPECIFIC OBJECTIVES AND RESULTS

1. To retrospectively evaluate the diagnoses of *L. infantum* infection in dogs with IBD.

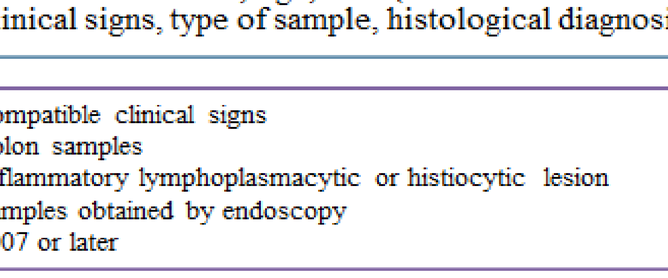

Figure 1. Percentages of *Leishmania* IHC performed in dogs diagnosed with IBD and percentages of *Leishmania* IHC results from Diagnet.

Inflammation pattern	Percentage
Lymphoplasmacytic enteritis	32.6 %
Lymphoplasmacytic colitis	19.4 %
Histiocytic colitis	2.6 %
Histiocytic enteritis	0.4 %

Table 1. Percentage of types of inflammation and the segment of the gastrointestinal tract affected in dogs with a diagnosis of IBD from Diagnet.

2. To determine *L. infantum* infection in colonic biopsies diagnosed with IBD and description of the clinical cases.

Detection of 1 positive case from 27 cases by IHQ technique (3.6 %).

Case	Breed	Sex	Age (years)	Presenting complaint	Endoscopic findings	Other observations
Dog 1	German Dogo	M	9	Large bowel diarrhoea	↑ Nematodes	Diagnosis of *Leishmania* 3 months before biopsy
Dog 2	Boxer	M	5	Chronic diarrhoea with hematochezia	-	Echography: mesenteric iliac lymphadenomegaly
Dog 3	Cocker Spaniel	M	15	Chronic hematochezia	-	-
Dog 4	Mixed breed	F	1	Chronic diarrhea with mucus and bleeding for 7 months. Anorexia for 3 days, apathy and weakness	Hypertrophy, hyperemia and mucosal petechial	Echography: alteration of duodenum and colon
Dog 5 (new +)	Belgian Shepherd	F c	12	Bloody diarrhea for 1 month	Duodenum and colon very altered with a friable and edematous mucosa	Diagnosed of *Leishmania* before biopsy by the clinical veterinarian

Table 2. Description of the clinical cases positive to *Leishmania* by IHC.

3. Description of the histological lesions and parasite load of cases diagnosed with IBD and infection with *Leishmania*.

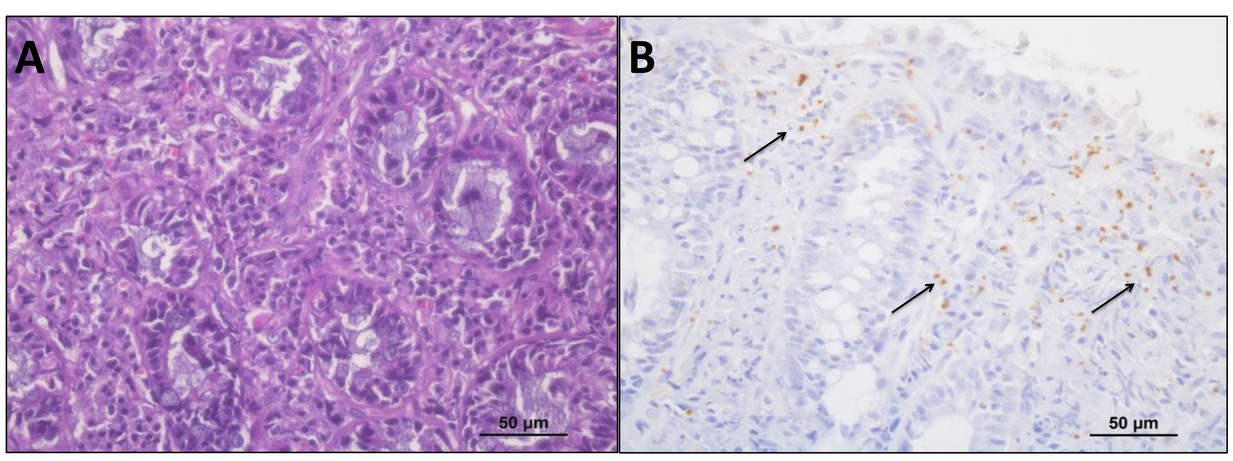

Figure 2. Positive case. Histological sections of the colon. A: intense histiocytic inflammation, HE staining. B: the same case than in the image A, but with IHC. Intense parasitation, amastigotes can be seen throughout the image. Arrows indicate some of amastigotes of *Leishmania* that can be seen.

Case	Year	Inflammation	Grade of intensity of inflammation	Amastigote count (counting 5 fields 40x)
Dog 1	1997	**lymphoplasmacytic**	Intense	33
Dog 2	2004	**lymphoplasmacytic**	Mild	37
Dog 3	2011	**Histiocytic**	Intense	15
Dog 4	2013	**Histiocytic**	Intense	Not able to count due to massive number of amastigotes
Dog 5 (new +)	2007	**lymphoplasmacytic**	Mild	2

Table 3. Description of IHC positive cases by *Leishmania*.

CONCLUSIONS

- The inflammation associated with *Leishmania* infection in the colon is both histiocytic and lymphoplasmacytic.
- Both the degree of inflammation and parasitism seems to vary between individuals.
- IHC for *Leishmania* is not commonly employed to describe this infection in dogs with a histological diagnosis of IBD.
- IHC for *Leishmania* should be routinely used as a diagnostic tool in endemic areas, to exclude or confirm an infection by this parasite in patients with a diagnosis of IBD.